Biological Control 56 (2011) 98–105

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Evaluation of herbivore-induced plant volatiles for monitoring green lacewings in Washington apple orchards

Vincent P. Jones [a,*], Shawn A. Steffan [a], Nik G. Wiman [a], David R. Horton [b], Eugene Miliczky [b], Qing-He Zhang [c], Callie C. Baker [a]

[a] *Department of Entomology, Tree Fruit Research and Extension Center, Washington State University, 1100 N. Western Ave., Wenatchee, WA 98801, United States*
[b] *USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, United States*
[c] *Sterling International Inc., 3808 N. Sullivan Rd., Bldg 16BV, Spokane, WA 99216-1630, United States*

ARTICLE INFO

Article history:
Received 17 June 2010
Accepted 6 October 2010
Available online 14 October 2010

Keywords:
HIPV
Squalene
Methyl salicylate
Iridodial
Chrysopa nigricornis
Chrysopa oculata
Chrysoperla plorabunda

ABSTRACT

We evaluated five herbivore-induced plant volatiles plus a male-produced pheromone as attractants for adult green lacewings in Washington apple orchards in 2008. We found at least five attractants or combinations of attractants were attractive to the three most abundant green lacewing species in our trials. *Chrysopa nigricornis* and *Chrysopa oculata* were attracted to the combination of methyl salicylate and iridodial with iridodial alone being the second best attractant. *Chrysoperla plorabunda* was found in lower numbers than *C. nigricornis* and *C. oculata*, but did exhibit a significant attraction to benzaldehyde. In mid-summer, we added the herbivore-induced plant volatile squalene to the study and found it to be exceedingly attractive, but only to male *C. nigricornis*. Whether alone or in combination, squalene attracted 4–5-fold more *C. nigricornis* than any other compound tested. Our data have revealed *C. nigricornis* to be an abundant orchard predator that can be readily monitored with squalene-baited traps. Despite the obvious promise of HIPVs in biological control programs, we urge caution in their deployment as large-scale attractants, at least until further studies have investigated potential disruption of natural enemy population dynamics.

1. Introduction

While sex pheromone based monitoring programs for key pests in agriculture or forest environments has been common for decades, the discovery and use of practical attractants for monitoring natural enemies has been a slower process that has focused on plant volatiles induced by herbivore feeding (known as herbivore-induced plant volatiles or HIPVs). Most of these compounds were discovered by chemical ecologists who compared volatile profiles released by herbivore-damaged plants to those of undamaged control plants; specific combinations of volatiles were then assayed in behavioral studies to determine activity (Dicke et al., 2003; Turlings et al., 1998). The literature on HIPVs shows that the ecological roles and behavioral interactions mediated by them is considerably broader and more complex than those mediated by sex pheromones alone (see reviews by Paré and Tumlinson (1999) and Vet and Dicke (1992)).

Studies of HIPVs in agricultural systems have recently increased in number, with the goal of using them as tools to induce host plant resistance and/or to concentrate natural enemies in areas for purposes of biological control (Gurr and Kvedaras, 2010; Kahn et al., 2008). Most studies have been preliminary steps towards field application, either by pairing a particular volatile (or mixture of them) with a trap to determine the types of natural enemies attracted, or by testing individual HIPVs in small plots to evaluate effects on natural enemy diversity and abundance (James, 2005; Lee, 2010). These approaches have been used to evaluate HIPVs as attractants in hops (James, 2003a,b, 2005; James and Price, 2004), grapes (James and Price, 2004), cotton (Williams et al., 2008; Yu et al., 2008), pear (Scutareanu et al., 1997), cherry (Toth et al., 2009), corn and sorghum (Kahn et al., 2008), and strawberry (Lee, 2010). From these studies and others, it is apparent that green lacewings (Neuroptera: Chrysopidae) as a group respond to a large number of HIPVs, but two attractants generally stand out in the recent literature. The first is the HIPV methyl salicylate (MS), which is considered to be attractive to a wide range of other natural enemies (James, 2003a, 2005, 2006). The second compound is iridodial, which is a male-produced male aggregation pheromone for certain lacewing species. Iridodial has been reported to attract (at various levels) several lacewing species including *Chrysopa oculata* Say, *Chrysopa nigricornis* Burmeister, *Chrysopa coloradensis* Banks, *Chrysopa quandripunctanta* Burmeister, and *Chrysopa septempunctata* Wesmael (Chauhan et al., 2007, 2004; Zhang et al., 2004, 2006a,b).

Another powerful lacewing attractant was accidentally discovered in early summer 2007 when one of us (NGW) evaluated

---

[*] Corresponding author. Fax: +1 509 662 8714.
E-mail address: vpjones@wsu.edu (V.P. Jones).

1049-9644/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2010.10.001

several HIPVs as possible attractants for adult tachinid parasitoids of obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae). In those evaluations, squalene-baited traps were found to be highly attractive to the lacewing *C. nigricornis* (NGW, unpublished). The choice of squalene in this initial screening was prompted by the work of Dutton et al. (2002, 2000), who showed that the chemical was released from golden delicious apples as the fruit was fed upon by the leafminer, *Phyllonorycter pomonella* Zeller (Lepidoptera: Gracillariidae), and showed also that the chemical attracted the leafminer parasitoid *Pholetesor bicolor* Ness (Hymenoptera: Braconidae). Squalene was provided to Curtiss (2008) (by NGW), who further evaluated it as an attractant in ecological studies of the movement of natural enemies into apple and pear orchards. Curtiss's studies showed the potential of the attractant as a monitoring tool and compared squalene to MS and a combination of MS + squalene. His results clearly showed that MS by itself had limited attraction for *C. nigricornis* compared to either squalene by itself or the combination of MS + squalene.

Our studies were designed to evaluate several promising HIPVs, the lacewing pheromone iridodial, and squalene as tools for monitoring abundance, diversity, and phenology of lacewings in tree fruits. This focus was chosen as a way to expand our understanding of the ecological role of the different natural enemy species and to enhance biological control through conservation and potentially spatial manipulation of natural enemy populations.

2. Materials and methods

2.1. Lure construction

We examined efficacy and release rates for six chemicals (Table 1). The products were selected on the basis of literature accounts suggesting that each might have some attractive properties to some natural enemies. Lures were constructed using 5 cm wide polyethylene tubing (PE) that were either 0.106 or 0.152 mm thick (Associated Bag Company, Milwaukee, WI). We examined two types of wicking material, either a 4 × 4 cm piece of polyester felt (Kunin Group, Hampton, NH), or a single 3.8 cm long dental wick (large size – 12.5 mm dia.; Patterson Dental, St. Paul, MN). Wick choice was based on the total volume of attractant needed for lure longevity; dental wicks provided a greater capacity to absorb attractants than the felt material, but the felt provided a greater surface area for release of volatiles at low volumes. Tubing length was 5 cm in length for lures containing the felt wicks and 7.5 cm in length for lures containing dental wicks. To construct the lures, we first heat-sealed one section of tubing at one end to create a small bag. We added a wick to the bag, then dispensed a single attractant on each wick at these rates: 0.5 ml per dispenser for benzaldehyde; 4 mg for iridodial; and 2 ml for all other materials. After the attractant was dispensed, the bag was heat-sealed at the open end. If combinations of attractants were to be used, the individual attractants were each placed in its own lure, and multiple lures were placed in each trap. We used white plastic delta traps (Trécé, Inc.; Adair, OK) to capture lacewings that approached the lures. The final size of the attractant-containing section of the lure lures; the 1.5 cm excess (i.e., unsealed) portion of the tubing was used to attach the lure to the delta trap. Once constructed, lures were stored in impermeable heat sealed metalized barrier pouches (Associated Bag Company, Milwaukee, WI) and frozen at −20 °C until use. Control lures were constructed the same way, but included distilled water in place of attractant.

2.2. Evaluation of lure release rates

Release rates for lures dispensing benzaldehyde, *cis*-3-hexene-1-ol, *cis*-3-hexenyl acetate, MS, squalene, and iridodial were determined for 3–4 weeks under field conditions. Lures were placed inside delta traps, and traps were placed in the canopy of large (3.5–4.5 m tall) apple trees in an orchard at the Washington State University Tree Fruit Research and Extension Center in Wenatchee, WA. Lures were periodically brought into the laboratory, weighed with an Ohaus Pioneer analytical balance (0.1 mg accuracy) (Pine Brooke, NJ) and returned to the field. Tests were run during the early spring (16 May to 13 June), summer (30 July to 27 August) and fall (3 October to 17 October) of 2008. We used four packets per lure/attractant combination and calculated average weight loss per day over a period extending to 28 d. If cumulative weight loss of a lure leveled out before the full 28 d had elapsed, we considered the lure to be depleted at the time the change occurred. We used linear regression to determine the relationship between cumulative weight loss and time; the slope of the regression provides an estimate the mean daily release rate. The longevity of the lure was calculated by dividing the total attractant weight by the average weight loss per day.

2.3. Long-term comparison of different attractants

The 2008 field studies were conducted using a randomized block design with the lures tested, orchard locations, test durations, and lure replacement intervals shown in Table 1. We had four blocks per orchard. Within each block, traps were separated by a minimum of 20 m; adjacent blocks were separated by at least 40 m. Treatments were randomly allocated among trap locations within blocks. Traps were placed 1.5–3 m high in the tree canopy

Table 1
Lures evaluated for lacewing attraction during summer 2008 in Central Washington apple orchards.

Attractants[a]	Source	Replacement interval (weeks)	Quincy	WSU-Sunrise	Moxee	Wenatchee Valley College
cis-3 Hexenyl acetate	Sigma–Aldrich W317101	3	8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
Benzaldehyde	Sigma–Aldrich 418099	1	8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
cis-3-Hexen1-ol	Sigma–Aldrich W256307	4	8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
Iridodial (IR)	Sterling International	4	8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
Methyl salicylate (MS)	Sterling International	4	8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
MS + IR	Sterling International		8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
Squalene (SQ)	Sigma–Aldrich S3626	4	18 July–9 October	–	–	–
SQ + MS			18 July–9 October	17 July–7 October	–	–
SQ + IR				17 July–7 October	–	–
SQ + IR + MS			18 July–9 October	–	–	–
Control			8 May–9 October	8 May–7 October	28 May–1 October	9 May–7 October
Orchard size (ha)			7.5	6.0	1.0	1.6

[a] Attractant combinations used separate lures of the same composition and source as the single component lures. All lures were made of 0.106 mm thick polyethylene tubing and used felt wicks as described in Section 2.

(generally in the top third of the canopy). The traps were rotated weekly to reduce location effects. During each rotation of the traps, the traps were moved to trees offset from trees previously containing a lure. This rotation minimizes chances that contamination of a tree (or induction of HIPVs) caused by the presence of an earlier lure would affect subsequent lure performance. Each experiment was dismantled and completely re-randomized monthly. Trap liners were removed each time the traps were checked, covered with saran wrap (S.C. Johnson & Son, Inc., Racine, WI), marked with location, block and lure type, and placed in a freezer until lacewings were identified and counted.

Trials were done at four orchards located in central and north-central Washington State: Moxee, Quincy, the Washington State University Sunrise Orchard (WSU- Sunrise) near Rock Island, and the Wenatchee Valley College (WVC) orchard in East Wenatchee. All of the orchards were used to evaluate the same seven lures or combination of lures (Table 1). Traps at the Moxee orchard were checked weekly, and traps at the other three locations were checked twice weekly.

Attractants that caught <20 lacewings at each location over the entire trial duration were excluded from statistical analysis. We also eliminated attractants that had a trap catch that was less than one-half of the control traps at a particular orchard. We censored the data because we were interested only in biologically active materials. For example, at the Moxee orchard (checked only once a week), we monitored 76 traps (4 reps × 19 weeks) over the season. Thus, a total of 20 lacewings collected over the duration of season provided an average of 0.26 lacewings per week, well below counts that would allow the attractant to be used as a monitoring tool. Even with this censoring, some of the remaining attractants were only weakly attractive. To overcome this problem, we analyzed lacewing numbers collected per trap summed across the entire trapping period, rather than weekly counts. Normality and homogeneity of variance was assessed in each analysis. When departures from assumptions occurred, we transformed the data (typically using $\log e(x + 0.5)$); if transformation failed to improve departures from normality and heterogeneity, we performed ANOVA on the ranks of the data. All post hoc tests used Tukey's HSD test to separate treatment means (Anonymous, 2009). Means were transformed back into the original units for presentation.

2.4. Mid-season squalene comparisons

The WSU-Sunrise and the Quincy blocks were large enough to test several squalene-based attractants. These tests began in mid-July (Table 1), once traps began to consistently collect lacewings. Squalene-based attractants were compared to the best performing materials of the long-term study, evaluated specifically over the period that the squalene-based lures were present (i.e., 17 July to 7 October at the WSU-Sunrise orchard and 18 July to 9 October at the Quincy orchard; Table 1). Data analysis was as described above (Section 2.3).

2.5. Effect of squalene lure age and wick type on trap captures

The longevity of the squalene lures was tested by aging lures in an orchard located in Quincy, WA. Lures were aged 1, 8, 15, 22, and 29 d before testing them for attractiveness. We tested both felt and dental wick dispensers (Section 2.1). All wicks were placed in 0.106 mm thick PE tubing using the methods described previously. Traps were checked every 3–4 d from 7 August to 19 August 2009.

Data were analyzed in two ways. First, we used a two-way ANOVA to evaluate the main effects of wick type, age of lure, and the interaction of lure age $*$ wick type for the first sampling period and the last sample periods. For the second analysis, we coded each lure by its relative age so that 1 was the youngest lure, 2 the next youngest, to the oldest lure. We then used a three-way ANOVA and evaluated the main effects of wick type, date sampled, relative lure age and all the two and three way interactions.

2.6. Effect of squalene release rate on trap captures

The release rates of the lures are generally a function of the PE tubing thickness and the surface area of the tubing that houses the wick. To reduce the release rate below that obtained with 0.152 mm PE tubing (the thickest available), we constructed a pouch composed of the 0.152 mm membrane heat-sealed along one side to an impermeable metalized "barrier" material (described in Section 2.1). This construction resulted in a release area of 50% smaller than provided by an unaltered pouch. To obtain even lower release rates, we constructed lures using the metalized barrier encompassing small "windows" of PE tubing, heat-sealed together.

We were unable to estimate release rates of the squalene lures gravimetrically, as weight loss was insignificant. Thus, to indirectly evaluate release rates for squalene, we conducted field bioassays using the different lure constructions. The first trial was run from 29 June to 24 July 2009 and consisted of five treatments: a standard 0.106 mm thick PE lure with 1 ml of squalene, 0.152 mm thick PE tubing + foil lure, and grey halo butyl rubber septa (West Pharmaceutical Services, Lionville, PA) dosed with either 1, 10, or 100 μl of squalene diluted in an equal volume of hexane. The septa were placed in a fume hood for 7 d to allow the squalene to be absorbed completely into the septa. The second trial was done from 24 July to 19 August 2009 and included the same first two lure formulations, but with the septa replaced by foil lures having a 0.106 mm thick PE window that was 0.5, 1.0, or 1.5 cm wide by 3.8 cm long; all lures had 1 ml of squalene added. The lures were placed in an orchard in Quincy, WA using the field protocols discussed Section 2.3.

2.7. Selectivity of attractants

Selectivity of the attractants was evaluated by determining the relative abundance of each species associated with each attractant, calculated as percentage of the total lacewing capture composed of each species. The data were collected as part of the experiments in Sections 2.3 and 2.4. We did not test if there were differences in the proportion of the three different lacewing species captured between attractants because we do not have accurate estimates of the relative abundance of each species that was independent of the attractant traps. Instead, we were interested in the relative selectivity of the different attractants at each location.

2.8. Sex ratio of *C. nigricornis* attracted to squalene-baited traps

We also examined the sex ratio of *C. nigricornis* caught in the squalene traps in 2008. We determined sex of 2119 lacewings on a total of 200 traps collected 13 June to 28 August at an orchard in the Quincy area. Trapping protocols (trap height, spacing, servicing methods) were the same as those described for experiments in Section 2.3. In 2009, we expanded this study to evaluate sex ratio of lacewings caught on traps in which squalene or the mixture of MS + squalene were used as attractants. This trial was done to assess whether sex ratio changed with addition of MS to squalene. We sampled a commercial orchard in the Quincy area from 31 July to 28 August and determined the sex in 4195 specimens (1959 from the squalene traps, 2154 from the MS + squalene traps). Trapping protocols were the same as described in Section 2.3.

Table 2
Release rates of various attractants determined by weight loss over time in the field at different times in 2008.

Attractant	PE tubing thickness (mm)	Lure load (ml)	Spring		Summer		Fall	
			Release rate (mg/day ± SEM)	Longevity (d)[a]	Release rate (mg/day ± SEM)	Longevity (d)[a]	Release rate (mg/day ± SEM)	Longevity (d)[a]
Benzaldehyde	0.106	0.5	67.3 ± 5.2	7.4	61.4 ± 3.7	8.1	24.0 ± 0.5	20.8
	0.152		53.0 ± 3.5	9.4	57.4 ± 3.0	8.7	15.4 ± 0.2	32.5
cis-3-Hexene-1-ol	0.106	2	7.9 ± 0.2	>50	12.0 ± 0.4	>50	*nt*[b]	*nt*[b]
	0.152		5.2 ± 0.1	>50	9.6 ± 0.3	>50	*nt*[b]	*nt*[b]
cis-3-Hexenyl acetate	0.106	2	139.2 ± 9.4	14.4	–[c]	<7	83.6 ± 1.6	23.9
	0.152		105.2 ± 2.4	19.0	–[c]	<7	58.1 ± 1.5	34.4
Methyl salicylate	0.106	2	51.1 ± 0.5	39.1	175.3 ± 4.4	11.4	56.7 ± 1.4	35.3
	0.152		*nt*[b]	*nt*[b]	116.2 ± 1.8	17.2	39.6 ± 0.9	>50
Mean temperature (°C ± SD)			17.6 ± 3.4		22.0 ± 3.4		10.2.4 ± 2.7	
Period			16 May–13 June		30 July–27 August		3 October–17 October	

[a] Amount of attractant in lure/release rate; longevities >50 d not reported.
[b] Not tested during this period.
[c] Lure was completely depleted at first check interval.

2.9. Lacewing identification

Lacewings were identified using the keys in Brooks (1994), Brooks and Barnard (1990), and Penny et al. (2000). Representative specimens have been stored as vouchers in 70% ETOH at the WSU-Behavior and Ecology lab in Wenatchee, WA.

3. Results

3.1. Lure release rates

Tests with the iridodial and squalene lures did not show sufficient weight loss to calculate release rates. However, for the remaining lures the release rate of each attractant was linear until depleted. The release rates were typically highest in the summer, followed by the spring, and then the fall, most likely because of the different temperature profiles during those periods (Table 2). The *cis*-3-hexene-1-ol lures had longevities >1 month at both bag thicknesses and overall testing periods, so that release rates could be readily controlled. Benzaldehyde is probably the most problematical of the lures, because it underwent a slow change from liquid to crystalline form starting ≈5–7 d after placement of lures in the field; at that point, the release rates lowered dramatically. This change in state caused us to replace those lures at 1-week intervals throughout our experiments. Increasing the volume or lure thickness did not seem to affect the rate of crystallization (data not shown). The *cis*-3 hexenyl acetate lure released very quickly, especially during the summer when the lures were found to be depleted the first time they were checked (3–4 d old). During the fall and spring, 0.152 mm thick lures of *cis*-3-hexenyl acetate had longevities of 19 d (spring) and 34.4 (fall). Release rates by the MS lures were ≈3–3.4-fold higher in summer than spring and fall (Table 2).

3.2. Long-term comparison of different attractants

Species composition of lacewings on traps varied among orchards. *C. nigricornis* was easily the most abundant lacewing species captured at the three orchards in north-central Washington and accounted for 85%, 72%, and 87% of the total lacewings at the Sunrise, Quincy, and WVC sites, respectively (Table 3). At the Moxee site, *C. oculata* comprised 56% and *C. nigricornis* 40% of the captures. At the other three orchards, *C. oculata* was the second most common species at WVC and Quincy and roughly equal in numbers to *Chrysoperla plorabunda* (Fitch) at the WSU-Sunrise orchard. *C. plorabunda* was caught in numbers only at the WSU-Sunrise and Quincy orchards (Table 3).

Table 3
Total number of green lacewings caught at four orchards with seven different attractants during summer 2008.

Location	*C. oculata*	*C. nigricornis*	*C. plorabunda*
Moxee	895	635	64
Sunrise	63	1305	175
Quincy	277	1056	145
Wenatchee Valley College	255	2157	64

The attractants *cis*-3 hexenyl acetate, benzaldehyde, and *cis*-3-hexen-1-ol caught only 16 *C. oculata* summed over all sites, thus these compounds were dropped from further analysis for this species. The largest numbers of captures occurred in the iridodial (619 lacewings) and MS + iridodial (1252 lacewings) treatments. MS by itself appeared to be nearly inactive with only 32 *C. oculata* caught over the trapping periods in the four orchards. We analyzed statistically the iridodial, MS + iridodial, MS, and water treatments to evaluate the effects of all possible combinations of MS and iridodial lures (Fig. 1A). The data were highly non-normal because of the low trap catches in the water and MS treatments and could not be transformed, so that we used the ranked ANOVA. In all four orchards, the attractant effect was highly significant, with the number captured always MS + iridodial > iridodial > MS > water. In three of the orchards, all materials were statistically different from each other. In the fourth orchard (Quincy), the MS and water treatments were not significantly different (Fig. 1A).

Numbers of *C. nigricornis* collected in the *cis*-3-hexen-1-ol or benzaldehyde baited traps were very low, thus these compounds were dropped from statistical analysis. The MS + iridodial treatment was again the best numerically at all locations, but was statistically better than iridodial in only one orchard and better than MS in only three of the four orchards (Fig. 1B). MS by itself and *cis*-3 hexenyl acetate were statistically better than the control in one orchard each.

The low numbers of *C. plorabunda* on traps made it difficult to evaluate the attractants (Fig. 1C). We found benzaldehyde had the highest number of captures in all four orchards, with MS, MS + iridodial, and the controls following (Fig. 1C). At the Sunrise orchard the benzaldehyde treatment was statistically better than the MS and MS + iridodial treatments. At the other locations, MS was generally the second best material and was not statistically different than the benzaldehyde treatment.

3.3. Mid-season squalene comparisons

Squalene + MS and squalene + iridodial were added to the Sunrise orchard on 17 July 2008 and squalene, squalene + MS,

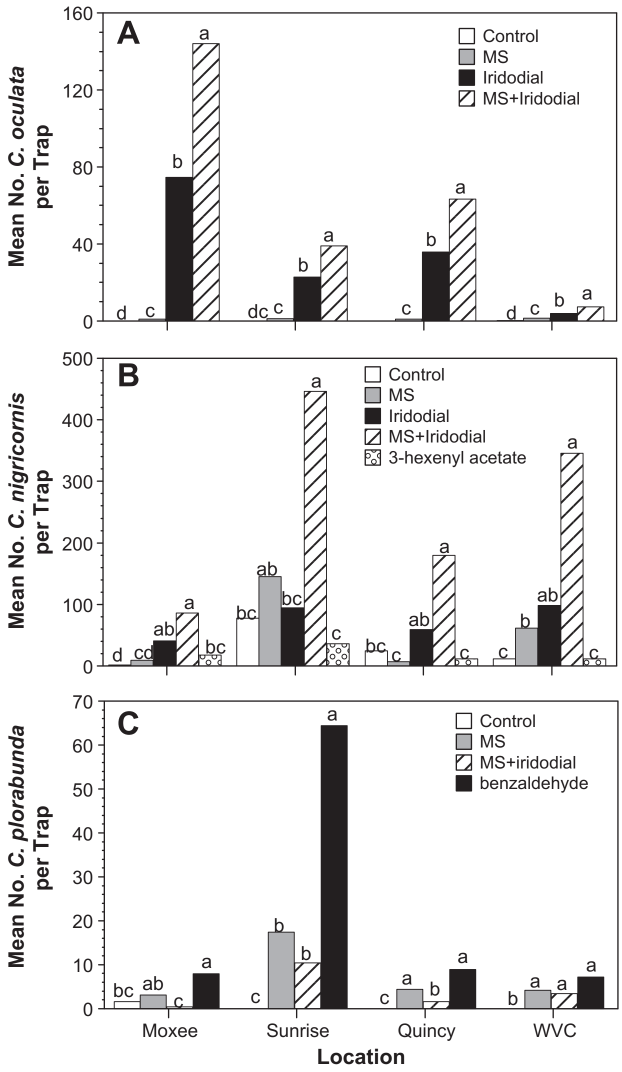

Fig. 1. Long-term attractant evaluations at four different orchards in Washington during 2008. (A) *Chrysopa oculata*, (B) *Chrysopa nigricornis*, and (C) *Chrysoperla plorabunda*. Numbers are mean captures per trap over the entire trapping period. Letters indicate significant differences within a location according to ANOVA based on ranks and Tukey's HSD test.

and squalene + iridodial + MS were added to the Quincy orchard on 18 July 2008. Evaluation of the mean trap catch from those periods to the end of the season at each orchard clearly demonstrated that squalene was extremely attractive to *C. nigricornis* either alone or when found in combination with MS or iridodial (Fig. 2A and B). At the Sunrise orchard, we caught an average of 10.4 and 23.5 *C. nigricornis* per trap day, respectively, for squalene + iridodial and squalene + MS over the 82 d the traps were in the field. At the Quincy orchard, we caught an average of 8.0, 7.6, and 8.6 *C. nigricornis* per trap day, respectively, for the squalene, squalene + MS, and squalene + iridodial + MS lures over the 83 d traps were in the field.

3.4. Effect of squalene lure age and lure type

The analysis on the first sampling interval showed no significant differences occurred by wick type ($p = 0.90$, $df = 1, 30$), lure age ($p = 0.11$, $df = 4, 30$) or the interaction of wick type $*$ lure age ($p = 0.17$, $df = 4, 30$). The last sampling interval showed similar trends with no significant differences found in any of the three fac-

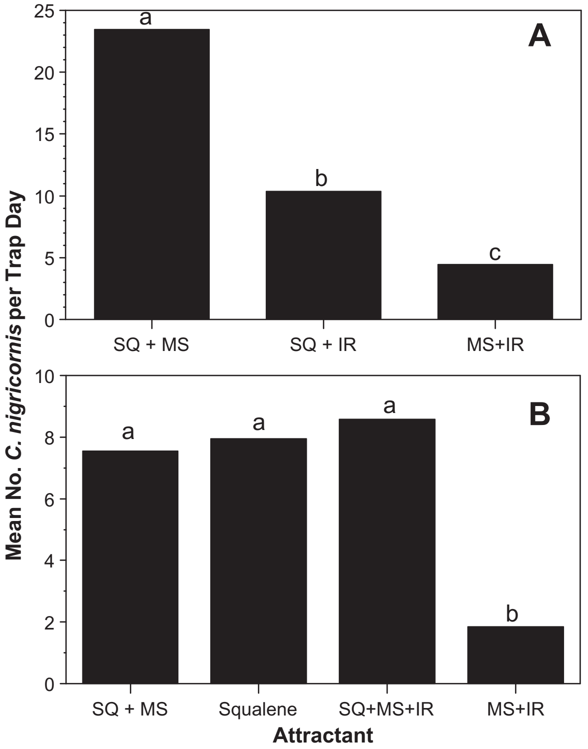

Fig. 2. Mean capture per trap day of *C. nigricornis* from mid-July to early October 2008 for the best non-squalene attractant and those containing squalene at (A) WSU-Sunrise and (B) Quincy. SQ, squalene; MS, methyl salicylate; and IR, iridodial. Bars with the same letters indicate significant differences within a location according to ANOVA based on ranks and Tukey's HSD test.

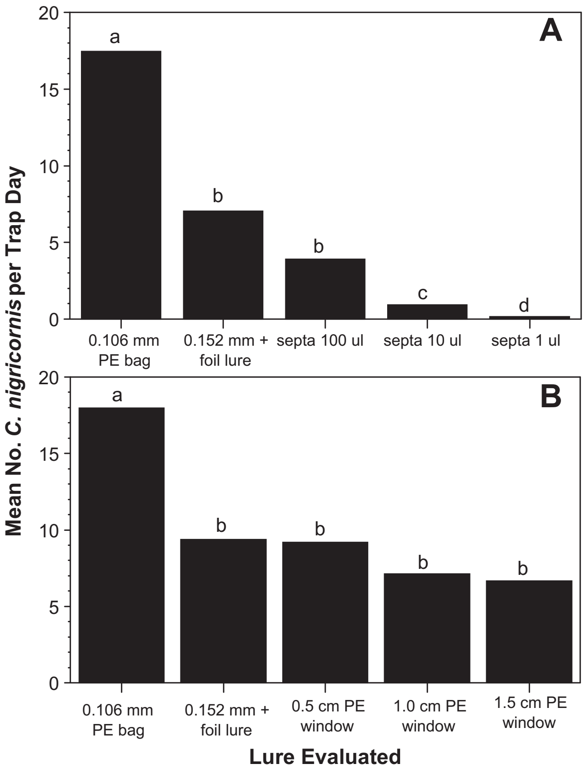

Fig. 3. Effect of different lure types on trap catch of *C. nigricornis* during summer 2009. (A) 10 July to 24 July. (B) 24 July to 19 August. Bars with the same letters indicate no significant differences according to ANOVA and Tukey's HSD test.

tors tested. Analysis on the entire data set showed that the only significant effect was the sampling date ($p < 0.0001$, $df = 4, 150$). Thus over the entire course of the experiment, lure age out to 44 d did not affect trap capture and there were no significant differences related to wick type.

3.5. Squalene dose effects

The first trial showed an increasing dose–response with increasing trap catch as the septa load varied from 1 to 100 μl (Fig. 3A). The 100-μl septa load was significantly poorer than the 0.106 mm thick PE tubing lure, but not significantly different than the 0.152 mm PE tubing + foil lure. Although we were able to create the 100-μl septa lures for this study, absorption required a 1 week period, which makes this loading impractical for anything but experimental purposes.

The second test showed that trap catch could be lowered by use of foil packets with small windows of PE tubing added (Fig. 3B). There were no significant differences between any of the foil bag treatments, suggesting that the easiest way to reduce lure efficiency is to use a 0.152 mm PE tubing + foil lure, which still caught significant numbers of *C. nigricornis*, but is easier to make than the lures with the smaller size windows.

3.6. Sex ratio of *C. nigricornis* collected on traps baited with squalene

Of the 2119 specimens of *C. nigricornis* collected in 2008, 2037 were male and 82 were female (3.3% female). A 2 × 2 contingency table analysis showed that female and male distributions were not independent ($G = 10.96$, $df = 1$, $p = 0.001$). Females occurred only on traps that had also captured at least four males.

In our comparisons of sex ratios on traps baited with squalene versus squalene + MS, we captured 4 females out of 1959 total captures of *C. nigricornis* on the squalene traps (0.21%) and 79 females out of 2154 *C. nigricornis* (3.5%) on the squalene + MS baited traps. These two sex ratios were significantly different ($G = 74.4$, 1 $df = 1$, $p < 0.001$). Females were captured only on traps that had collected a minimum of 21 and 28 males for the squalene and squalene + MS baited traps, respectively.

3.7. Selectivity of the lures for different lacewings

Relative capture rates of the three species among the different lures were tabulated for each location (Table 4). It was apparent that lures containing squalene attracted primarily *C. nigricornis* (14,472 caught), with only 94 *C. oculata* and 18 *C. plorabunda* trapped. This result may in part be due to the relative size of the

Table 4
Selectivity of different attractants to three lacewing species collected in Washington apple orchards in summer 2008.

Location	Attractant	% *C. oculata*	% *C. nigricornis*	% *C. plorabunda*	N
Moxee					
	cis-3 Hexenyl acetate	12.5	83.0	4.5	88
	Benzaldehyde	5.6	5.6	88.9	36
	cis-3-Hexen-1-ol	25.0	0.0	75.0	4
	Iridodial (IR)	64.2	35.2	0.6	466
	Methyl salicylate (MS)	8.6	69	22.4	58
	MS + IR	62.4	37.4	0.2	925
	Control	0.0	58.8	41.2	17
	Total				1594
Quincy					
	cis-3 Hexenyl acetate	0.0	98.0	2.0	51
	Benzaldehyde	0.0	32.1	67.9	53
	cis-3-Hexen-1-ol	0.0	0.0	0.0	0
	IR	37.7	62.3	0.0	382
	MS	9.6	55.8	34.6	52
	MS + IR	25.8	73.4	0.7	983
	Squalene (SQ)[a]	0.1	99.9	0.0	2849
	SQ + MS[a]	0.0	99.9	0.0	2511
	SQ + MS + IR[a]	2.5	97.1	0.3	3152
	Control	0.0	100	0.0	102
	Total				10,135
WSU-Sunrise					
	cis-3 Hexenyl acetate	0.0	93.6	6.4	78
	Benzaldehyde	0.5	29.0	70.5	183
	cis-3-Hexen-1-ol	0.0	80.0	20	30
	IR	17.9	81.3	0.9	235
	MS	2.4	87.2	10.4	335
	MS + iridodial	12.6	85.4	2.0	1047
	SQ + IR[b]	0.4	99.5	0.1	2096
	SQ + MS[b]	0.1	99.8	0.1	3866
	Control	0.6	99.4	0.0	157
	Total				8027
Wenatchee Valley College					
	cis-3 Hexenyl acetate	0.0	96.1	3.9	51
	Benzaldehyde	0.0	45.3	54.7	53
	cis-3-Hexen-1-ol	0.0	100	0.0	4
	IR	18.8	80.8	0.4	490
	MS	2.2	91.5	6.3	272
	MS + IR	10.1	89.0	0.9	1556
	Control	0.0	100	0.0	50
	Total				2476
	Grand total				22,232

[a] Only out from 18 July to end of test.
[b] Only out from 17 July to end of test.

different lacewing populations in each orchard, however, relatively large numbers of *C. oculata* were found in both the iridodial and iridodial + MS lures in these orchards. Thus, it appears that squalene has limited attraction to *C. oculata* unless it was mixed with iridodial. Iridodial attracted relatively high numbers of *C. nigricornis* as well as *C. oculata*, so that use of iridodial along with squalene in a monitoring program would require identification of lacewings to species. *C. plorabunda* was most commonly found in the benzaldehyde containing lures.

4. Discussion

Our data show that we can easily monitor populations of all three lacewing species common in Washington apple orchards. *C. plorabunda* was captured only in low numbers in our studies, but this may be due to a number of different factors: the need for a better lure, different seasonal phenology (our recent tests show it emerges very early in the season compared to the other two species), differential susceptibility to pesticides, or simply because it is less abundant than the other two species.

The release rate and longevity of the lures used for the various attractants are key factors that need further research. It is clear that the 0.106 mm thick PE tubing lure with 2 ml of *cis*-3-hexenyl acetate became depleted during the summer well before we changed the lures. Thus, our lack of response to this lure should be viewed with caution. Recent studies (V.P.J. and C.C.B., unpublished) show that a higher initial load of the attractant (3 ml) and use of a 0.152 mm PE tubing + foil lure controls release rates during the summer and lasts >1 month. While we were able to regulate most of the other compounds well, there is no guarantee that the release rates we achieved were optimal for attracting these lacewing species. Having accurate estimates of release rates will be crucial for comparison of effects among different studies and lacewing species.

Our results with MS, iridodial and the other HIPVs mirror many of the results reported by Zhang et al. (2006a). MS by itself appeared to have no attraction for *C. oculata*, and was only statistically better than the control in one orchard for *C. nigricornis*. Iridodial was attractive to both *C. oculata* and *C. nigricornis*, but the combination of MS + iridodial was statistically more attractive than either iridodial or MS alone. These results appear to conflict with other studies in Washington State, where James (2003a, 2006) found MS was attractive to both *C. nigricornis* and *C. oculata*. We cannot explain the differences other than to note the likely differences in release rates between James' studies and ours; our studies (and those of Zhang et al. (2006a)) used the PE tubing lures, while James' studies used glass vials with cotton loosely packed in the opening.

Our studies showed that squalene is a highly attractive semiochemical that provided nearly species and sex-specific attraction of male *C. nigricornis*. Female *C. nigricornis* were never found on traps without >4 males present, which suggests that the females are being attracted (weakly) to the males (either chemically, visually, or acoustically). The cause of the low female capture may be similar to that observed with *C. oculata* attraction to iridodial, where females were found attracted to the vicinity of traps but rarely entered them (Chauhan et al., 2007); studies are ongoing to investigate whether female *C. nigricornis* exhibits a similar response to squalene-baited traps.

The extreme attractiveness of squalene to *C. nigricornis* is also notable when compared to any of the materials reported for a variety of natural enemies either in the literature or from the MS, iridodial or the combination of MS and iridodial in our trials. Mean trap capture per trap day easily exceeded 20 adult male *C. nigricornis* over long periods of time. While the high trap capture is impressive and provides us with an excellent tool with which to track *C. nigricornis* population density and phenology, it is also clear that lower release rate lures may be necessary to prevent removal of *C. nigricornis* in such numbers that its population dynamics are affected. Our studies of release rates provide several lure formulations that can either reduce or increase the trap sensitivity; the longevity studies show that all of them were stable for at least 44 d.

Although some may see squalene as a perfect candidate for manipulating spatial distribution of *C. nigricornis* in an attempt to maximize predation in the tree canopy, there are many potential pitfalls with this approach. First, if the attractant is uniformly distributed throughout the orchard, it may reduce predator efficiency at low prey densities if *C. nigricornis* were no longer able to use herbivore-induced squalene production to narrow its search area. This effect may actually increase the overall prey density, resulting in greater economic damage. Secondly, if females are not attracted at least near to the locations where squalene lures are placed, then the lures may disrupt the mate finding process, which would reduce numerical response in subsequent generations. Third, *C. nigricornis* appears to be primarily found in the tree canopy (Horton et al., 2009), so that cover crops are unlikely to be a high-density source that can be used in a push–pull type strategy. Thus, assuming a long-range attraction (of which we have no proof at this point), movement of *C. nigricornis* from adjacent blocks will result in a "robbing Peter to pay Paul" scenario. If the attraction is much more local, then manipulating spatial distribution may have practical applications (if the other objections in this paragraph are satisfied). Our objections to use of squalene are primarily against its use as a season-long tool to concentrate *C. nigricornis.* We do see benefits to aggregating the population for shorter periods of time (e.g., 1–2 weeks) to augment functional and numerical responses to help suppress pest populations in a small portion of the environment or to move populations to areas that will not be subject to pesticide treatments.

Irrespective of the use of squalene as a tool to change spatial or temporal aggregation in the orchard, having a lure that attracts natural enemies reliably has broad consequences for studies of population dynamics and for implementing conservation biological control tactics in agricultural and forestry environments. In particular, the implementation of biological control is hindered by many IPM consultants' perception that it is ineffective, at least in part because of the inefficiency of normal sampling methods (e.g., beat trays). For example, we sampled five commercial orchards 2–3 times a week using beat trays from 1 March to mid-October 2009 and captured only 12 adult *C. nigricornis*. In the same orchards during the same time period, four squalene traps captured a total of 25,604 adult *C. nigricornis*. The large number of *C. nigricornis* captured in the traps and the ability to detect changes in adult population levels before and after treatment should provide a powerful tool for that will help IPM practitioners to understand the role of *C. nigricornis* in biological control and its sensitivity to different management programs.

Acknowledgments

The field assistance of Teah Clement, Cameron Aguilar, Kodi Jaspers, Stacey McDonald, and Ivan Arroyo is gratefully acknowledged. This research was funded in part by grants to V.P.J. and D.R.H. from the Washington State Tree Fruit Research Commission and USDA-NIFA SCRI Grant No. 2008-04854.

References

Anonymous, 2009. JMP Statistics and Graphics Guide. Version 8.02. SAS Institute Inc., Cary, NC.

Brooks, S.J., 1994. A taxonomic review of the common green lacewing genus *Chrysoperla* (Neuroptera: Chrysopidae). Bulletin British Museum (Natural History) Entomology 63, 137–210.

Brooks, S.J., Barnard, P.C., 1990. The green lacewings of the world: a generic review (Neuroptera: Chrysopidae). Bulletin British Museum (Natural History) Entomology 59, 117–286.

Chauhan, K.R., Levi, V., Zhang, Q.-H., Aldrich, J.R., 2007. Female goldeneyed lacewings (Neuroptera: Chrysopidae) approach but seldom enter traps baited with the male-produced compound iridodial. Journal of Economic Entomology 100, 1751–1755.

Chauhan, K.R., Zhang, Q.-H., Aldrich, J.R., 2004. Iridodials: enantiospecific synthesis and stereochemical assignment of the pheromone for the golden-eyed lacewing, *Chrysopa oculata*. Tetrahedron Letters 45, 3339–3340.

Curtiss III, R.T., 2008. Attractiveness of Semiochemicals to Green Lacewings for Biological Control in Pome Fruit. MS Thesis. Washington State University, Pullman, 83pp.

Dicke, M., de Boer, J.G., Hofte, M., Rocha-Granados, M.C., 2003. Mixed blends of herbivore-induced plant volatiles and foraging success of carnivorous arthropods. Oikos 101, 38–48.

Dutton, A., Mattiacci, L., Amado, R., Dorn, S., 2002. A novel function of the triterpene squalene in a tritrophic system. Journal of Chemical Ecology 28, 103–116.

Dutton, A., Mattiacci, L., Dorn, S., 2000. Plant-derived semiochemicals as contact host location stimuli for a parasitoid of leafminers. Journal of Chemical Ecology 25, 2259–2273.

Gurr, G.M., Kvedaras, O.L., 2010. Synergizing biological control: scope for sterile insect technique, induced plant defences and cultural techniques to enhance natural enemy impact. Biological Control 52, 198–207.

Horton, D.R., Jones, V.P., Unruh, T.R., 2009. Use of a new immunomarking method to assess movement by generalist predators between a cover crop and tree canopy in a pear orchard. American Entomologist 55, 49–56.

James, D.G., 2003a. Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, *Chrysopa nigricornis*. Journal of Chemical Ecology 29, 1601–1609.

James, D.G., 2003b. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. Environmental Entomology 32, 977–982.

James, D.G., 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. Journal of Chemical Ecology 31, 481–495.

James, D.G., 2006. Methyl salicylate is a field attractant for the goldeneyed lacewing, *Chrysopa oculata*. Biocontrol Science and Technology 16, 107–110.

James, D.G., Price, T.S., 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. Journal of Chemical Ecology 30, 1613–1628.

Kahn, Z.R., James, D.G., Midega, C.A.O., Pickett, J.A., 2008. Chemical ecology and conservation biological control. Biological Control 45, 210–224.

Lee, J.C., 2010. Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. Environmental Entomology 39, 653–660.

Paré, P.W., Tumlinson, J.H., 1999. Plant volatiles as a defense against insect herbivores. Plant Physiology 121, 325–331.

Penny, N.D., Tauber, C.A., De Leon, T., 2000. A new species of *Chrysopa* from Western North America with a key to North American Species (Neuroptera: Chrysopidae). Annals of the Entomological Society of America 93, 776–784.

Scutareanu, P., Drukker, B., Bruin, J., Posthumus, M.A., Sabelis, M.W., 1997. Volatiles from *Psylla* infested pear trees and their possible involvement in attraction of anthocorid predators. Journal of Chemical Ecology 23, 2241–2261.

Toth, M., Szentkiralyi, F., Vuts, J., Letardi, A., Tabilor, M.R., Jaastad, G., Knudsen, G.K., 2009. Optimization of a phenylacetaldehyde based attractant for common green lacewings (*Chrysoperla carnea s. l.*). Journal of Chemical Ecology 35, 449–458.

Turlings, T.C.G., Bernasconi, M.L., Bertossa, R., Bigler, F., Carloz, G., Dorn, S., 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. Biological Control 11, 122–129.

Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37, 141–172.

Williams III, L., Rodriguez-Saona, C., Castle, S.C., Zhu, S., 2008. EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. Journal of Chemical Ecology 34, 1190–1201.

Yu, H., Zhang, Y., Wu, K., Gao, X.W., Guo, Y.Y., 2008. Field-testing of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. Environmental Entomology 37, 1410–1415.

Zhang, Q.-H., Chauhan, K.R., Erbe, E.F., Vellore, A.R., Aldrich, J.R., 2004. Semiochemistry of the goldeneyed lacewing *Chrysopa oculata*: attraction of males to a male-produced pheromone. Journal of Chemical Ecology 30, 1848–1870.

Zhang, Q.-H., Schneidmiller, R.G., Hoover, D.R., Young, K., Welshons, D.O., Margaryan, A., Aldrich, J.R., Chauhan, K.R., 2006a. Male-produced pheromone of the green lacewing, *Chrysopa nigricornis*. Journal of Chemical Ecology 32, 2163–2176.

Zhang, Q.-H., Sheng, M., Chen, G., Aldrich, J.R., Chauhan, K.R., 2006b. Iridodial: a powerful attractant for the green lacewing *Chrysopa septempunctata* (Neuroptera: Chrysopidae). Naturwissenschaften 93, 461–465.